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FINAL REPORT

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National Aeronautics and Space Administration

Title: Role of Thyroxine in Space-Developed Jellyfish

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INTRODUCTION

The *Aurelia* Metamorphosis Test System was previously used to determine the effects of the space environment on the development and behavior of tiny (1-2 mm) jellyfish ephyrae during the SLS-1 and IML-2 missions. Results from the SLS-1 experiment included the discovery that statolith numbers were significantly reduced in Earth-formed ephyrae flown for nine days in space as compared with ground-based controls. In addition, upon return to Earth, six times more ephyrae which had developed in space than those developed on Earth had pulsing abnormalities, indicating that either these animals did not form their neuromuscular structures normally while in space or they were unable to adapt to the 1g environment upon return to Earth.

The metamorphosis process, which enables the formation of ephyrae from polyps is influenced by a hormone, Jf-T4 (jellyfish thyroxine) which is synthesized following iodine administration. Two groups of polyps in space, however, formed ephyrae without iodine administration indicating that Jf-T4 synthesis, utilization, or excretion was different in the ephyrae. Increased synthesis or build-up in the media of the hormone may also be linked to the increased demineralization of statoliths found in space-exposed ephyrae. In previous experiments, we found that externally administered thyroxine causes increased demineralization of statoliths on Earth.

Abnormal pulsing in ephyrae following return to Earth during the SLS-1 mission may also be traced to increased Jf-T4 levels. Thyroxine is known to be important to the normal development and function of the nervous system, heart, and skeletal muscles in higher animals.

For this third Jellyfish-in-Space experiment, we proposed to quantitate the levels of Jf-T4 and of T4 receptors in space-developed ephyrae and media and to compare these levels with those of animals developing and at 1g in space and on Earth. We expected to be able to determine whether Jf-T4 synthesis and/or secretion is different in space-flown jellyfish than in controls and to determine which cells (nerve, muscle, lithocytes, etc.) may have enhanced Jf-T4 levels. However, NASA deselected this experiment in August, 1997.

FINAL PROGRESS REPORT- Sept 19, 1995 - Sept 18, 1997

During the two years (Sept 19, 1995-Sept 18, 1997) that this project was funded, research emphasis was placed almost exclusively on preparing the jellyfish for a shuttle flight which was selected by NASA to be flown in 1998 using the ARF (Aquatic Research Facility) designed by the Canadian Space Agency. To this end, we developed the Experiment Description and Scientific Requirements, the Ground Support Requirements, and the Crew Training Manual. The ECP was developed by the Canadian Space Agency and the jellyfish experiment was programmed into the ARF. The P.I. attended all of the IWGs (Investigator Working Groups) in Ottawa and at KSC where she presented results of biocompatibility testing of the SCUs of the ARF and of fixation results in the SCUs. She and her technicians were trained by Canadian engineers at her laboratory to assemble the SCU's. The experiment was ready for the SVT in October of 1997 when it was deselected in August. Experiments done to achieve this high level of readiness in two years are described below.

Biocompatibility of the SCUs. Twenty-nine tests using a total of 2068 jellyfish at 22°C were done to (1) to determine the number of polyps which can metamorphose in an SCU and give rise to normal ephyrae. The Metamorphosis Test System was used to evaluate the biocompatibility of the SCUs. Each ephyra was examined microscopically and

the numbers of arms, rhopalia, statoliths/rhopalia were recorded. In addition, the number of pulses per minute and the swimming behavior of each animal was recorded; (2) to determine the highest number of polyps which will begin segmenting prior to metamorphosis (time of maximum hormone production) and the number of ephyrae which will survive (and demineralize) for 11 days without feeding in the SCUs.

Final results of these experiments were: Thirty iodine-treated polyps per SCU which could develop into ephyrae in micro-g could be flown. 150 polyps per SCU could be induced to metamorphose in space and would begin strobilation three days later. Two groups of 30 ephyrae per SCU could be flown for the demineralization studies.

Fixation in the SCUs: Additional tests and numbers of animals were used to determine the best fixation method in the SCUs so that the jellyfish could be fixed in space. The method of choice was determined to be injection of 1.1 ml of 50% glutaraldehyde (diluted from 70% glutaraldehyde with sodium cacodylate buffer) followed by the post-fixation treatment described above. All of these fixations were followed by post-fixation with 6% glutaraldehyde (simulated post-flight) buffer rinse, osmium post-fix, and tannic acid treatment prior to embedding the samples in Epon. Ultrathin sections of rhopalia and the neuromuscular system were viewed with the TEM following glutaraldehyde fixation, and post-fixation with osmium and tannic acid. This method provided the best fixation of both jellyfish rhopalia (especially hair cells) and muscle

A simulation was done in which fixative was injected into an SCA with strobilating jellyfish and the turbulence of the introduced fluid was videotaped. The jellyfish were fixed instantly and were not damaged by the streaming fixative. Likewise a simulation of KI induction which would have occurred in flight was done in which the KI was introduced into the fixative block and then injected into the SCA containing the jellyfish. The jellyfish were not damaged by the injection.

Jellyfish Cultures: Large numbers of jellyfish polyps were collected and tested for normality over this two year period so that healthy polyps which consistently gave rise to normal ephyrae were available for the SVT and, ultimately, for the flight experiment.

Jf-T4 isolation and purification: After testing numerous extraction methods, we have chosen 80% ethanol, 20 mM NaOH to extract the hormone from *Aurelia*. This solvent mixture has proved to extract and preserve more hormone than acidic ethanol, pronase treatment, or a number of other treatments. The alkaline ethanol mixture is homogenized in 80% ethanol and 20 mM NaOH to extract the T4. The solution is centrifuged at 10,000 g for 10 minutes. The supernatant is removed and the pellet is re-extracted two more times. The pooled supernatant is then diluted with aqueous HCl to a final concentration of 20% ethanol, 0.375 M HCl. The ethanol-HCl mixture is run over 3 ml beds of LH-20 resin, previously treated with 0.1 M HCl, contained in polyethylene columns with glass wool frits. The columns are washed consecutively with two 3 ml portions of 0.1 M HCl, 3 ml of distilled H₂O, 1.8 ml of ethanol:0.1 M NH₄OH, aqueous (1:1) and three 3 ml portions of 0.1 M NH₄OH in ethanol. The first portion of the 0.1 M NH₄OH in ethanol contains the iodinated compounds of interest. These fractions are combined and dried under nitrogen at 40°C. The compounds are then separated by HPLC. The HPLC conditions are a 250 mm Beckman Ultrasphere C18 column with 5 mM particles in a mobile phase of 40:30:10 (water:methanol:acetonitrile) with 5 mM phosphate, pH 3.5 at a flow rate of 1.1 ml. Timed fractions containing the compounds of interest are collected using iodinated standards as a reference. The LH-20 method has been used by us for both T4 RIA and Jf-T4 isolation. Note that the LH resin treatment will remove resident T4 and T3 (if present) for RIAs for T4 and T3 (the latter to verify previous TLC and HPLC radiochemical findings of a lack of T3 in *Aurelia*) which can then be performed on the LH-20 elution fractions.

PROGRESS -SEPT 19, 1997 - Sept 15, 1998
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Jellyfish Cultures: The numerous jellyfish cultures developed for the ARF jellyfish in space experiment were maintained in this time period so that they would be available in the event that funding would again become available from NASA. In addition, some polyps (including off-spring from polyps flown in the SLS-1 Jellyfish-in-Space Experiment) were maintained in a sterile nutrient medium and were induced to form ephyrae periodically. These animals were able to form ephyrae as long as eight years after their ancestor polyps were exposed to micro-g.

Immunolocation Jf-T4 receptors: A method was developed for the detection of all three subunits (alpha1, alpha2, and beta1) of the thyroid receptor in intact animals. We detected immuno-reactivity in ephyra cells in response to all three subunits, suggesting that the thyroid receptor is present in the jellyfish and possesses antigenic sites similar to those in mammalian thyroid receptors. Some rhopalia, including the touch-plate area, as well as manubria, and muscle areas were immunoreactive. Further, evidence suggests that these receptors are located in the nucleus and on the plasmalemmal membranes.

Publications:

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Abstracts:

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